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(54) Title: MODIFIED PEPTIDES AND PEPTIDOMIMETICS FOR USE IN IMMUNOTHERAPY

(57) Abstract: The invention relates to a modified peptide derived from H-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH having general formula (II): Q-A1-A2-A3-A4-A5-A6-A7-A8-A9-A10-A11-A12-A13-Z. In general formula (II), A1 through A13 correspond with the amino acids of formula (I), Q corresponds with H and Z corresponds with OH. The modifications according to the present invention are selected one or more of the groups a, b or c, consisting of a) substitution of 1-6, preferably 1-4 amino acids at A1 through A13 with non-natural amino acids or β amino acids; b) substitution of one or more amide bonds with reduced amide bonds or ethylene isosteres; c) substitutions at Q and/or Z and, optionally, d) substitution of natural amino acids up to a total of 6 modifications. The peptides can be used for inducing tolerance induction in patients suffering from autoimmune diseases.

Modified peptides and peptidomimetics for use in immunotherapy

The present invention relates to modified peptides which are based on HC gp-39 (263-275), pharmaceutical compositions comprising such peptides as well as the use of these peptides for inducing tolerance induction in patients suffering from autoimmune diseases.

The immune system is established on a principle of discrimination between foreign antigens (non-self antigens) and autoantigens (self antigens, derived from the individuals own body) achieved by a build-in tolerance against the autoantigens.

The immune system protects individuals against foreign antigens and responds to exposure to a foreign antigen by activating specific cells such as T- and B lymphocytes and producing soluble factors like interleukins, antibodies and complement factors. The antigen to which the immune system responds is degraded by the antigen presenting cells (APCs) and a fragment of the antigen is expressed on the cell surface associated with a major histocompatibility complex (MHC) class II glycoprotein. The MHC-glycoprotein-antigen-fragment complex is presented to a T cell which by virtue of its T cell receptor recognizes the antigen fragment conjointly with the MHC class II protein to which it is bound. The T cell becomes activated, i.e. proliferates and/or produces interleukins, resulting in the expansion of the activated lymphocytes directed to the antigen under attack (Grey et al., Sci. Am., 261:38-46, 1989).

Self antigens are also continuously processed and presented as antigen fragments by the MHC glycoproteins to T cells (Jardetsky et al., Nature 353:326-329, 1991). Self recognition thus is intrinsic to the immune system. Under normal circumstances the immune system is tolerant to self antigens and activation of the immune response by these self antigens is avoided.

When tolerance to self antigens is lost, the immune system becomes activated against one or more self antigens, resulting in the activation of autoreactive T cells and the production of autoantibodies. This phenomenon is referred to as autoimmunity. As the immune response in general is destructive, i.e. meant to destroy the invasive foreign antigen, autoimmune responses can cause destruction of the body's own tissue.

The contribution of T cells to autoimmune diseases has been established in several studies. In mice, experimental autoimmune encephalomyelitis (EAE) is mediated by a highly restricted group of T cells, linked by their specificity for a single epitope of myelin basic protein (MBP) complexed to an MHC class II molecule. In the Lewis rat, a species with high susceptibility to various autoimmune diseases, disease has been shown to be mediated by T cells. In humans autoimmune diseases are also thought to be associated with the development of auto-aggressive T cells.

A destructive autoimmune response has been implicated in various diseases such as rheumatoid arthritis (RA), in which the integrity of articular cartilage is destroyed by a chronic inflammatory process resulting from the presence of large numbers of activated lymphocytes and MHC class II expressing cells. The mere presence of cartilage appears necessary for sustaining the local inflammatory response: it has been suggested that cartilage degradation is associated with the activity of cartilage-responsive autoreactive T cells in RA (Sigall et al., Clin. Exp. Rheumat. 6:59, 1988; Glant et al., Biochem. Soc. Trans. 18:796, 1990; Burmester et al., Rheumatoid arthritis Smolen, Kalden, Maini (Eds) Springer-Verlag Berlin Heidelberg, 1992). Furthermore, removal of cartilage from RA patients by surgery was shown to reduce the inflammatory process (R.S. Laskin, J. Bone Joint Surgery (Am) 72:529, 1990). The cartilage proteins are therefore considered to be target autoantigens which are competent of stimulating T cells. Activation of these autoreactive T cells leads to development of autoimmune disease. However, the identification of the autoantigenic components that play a role in the onset of rheumatoid arthritis has so far remained elusive.

The inflammatory response resulting in the destruction of the cartilage can be treated by several drugs, such as for example steroid drugs. However, these drugs are often immunosuppressive drugs that are nonspecific and have toxic side effects. The disadvantages of nonspecific immunosuppression makes this a highly unfavourable therapy.

The antigen-specific, nontoxic immunosuppression therapy provides a very attractive alternative for the nonspecific immunosuppression. This antigen-specific therapy involves the treatment of patients with the target autoantigen or with synthetic T cell-reactive peptides derived from the target autoantigen. These synthetic peptides correspond to T cell epitopes of the autoantigen and can be used to induce specific T

cell tolerance both to themselves and to the autoantigen. Desensitization or immunological tolerance of the immune system is based on the long-observed phenomenon that animals which have been fed or have inhaled an antigen or epitope are less capable of developing a systemic immune response towards said antigen or epitope when said antigen or epitope is introduced via a systemic route.

Rheumatoid arthritis is an autoimmune disease that occurs more frequently in HLA-DR4-positive individuals. The disease association may indicate that DR4 molecules present autoantigens to T-cells. The target of this autoimmune disease is the joint where the articular chondrocyte presents a unique cell type producing products organized in a matrix. It is thought that joint destruction as seen in RA is mediated by cartilage-specific, autoreactive T-cells. The cartilage-derived protein Human Cartilage gp-39 (HC gp-39) has recently been identified as a candidate autoantigen in RA. A dominant epitope of the HC gp-39 protein, the peptide covering the 263-275 sequence, was preferentially recognized in RA patients suggesting that this epitope is a target of the autoimmune attack in rheumatoid arthritis. Eight out of 18 RA patients responded to this peptide and no responders were found in the healthy donor group (Verheijden et al., Arthtritis Rheum. 40:1115, 1997). Thus, the data strongly suggest that this peptide or the HC gp-39 protein is a target for immune recognition in the joint.

The significance of HC gp-39 for arthritic disease was further demonstrated by its arthritogenicity in Balb/c mice. A single injection in the chest region with µg amounts of protein mixed in IFA, induced a chronic joint inflammation reminiscent of RA.

The response to the HC gp-39 peptide 263-275 was further examined by generating a set of DRB1*0401-restricted, peptide-specific T-T hybridomas from DRB1*0401-transgenic mice following immunisation with HC gp-39. The fine specificity of the hybridomas specific for peptide 263-275 in the context of DR4 (DB1*0401) was defined and compared. As a result 3 hybridomas differing in their recognition of the 263-275 epitope presented by DRB1*0401 encoded molecules were identified. (The difference in epitope recognition between the three hybridomas used became visible when N- and C-terminal truncated peptides within the 263-275 sequence were used for stimulation of the different hybridomas). The 5G11 hybridoma was found to respond optimally to the 265-275 sequence. In contrast, recognition by the 8B12 hybridoma was centered around sequence 264-274 whereas the 14G11 hybridoma was optimally responsive to 264-275.

Tolerization of HC gp-39 (263-275)-reactive T-cells may be of benefit to RA patients. The present invention provides for modified peptide derivatives based on the HC gp-39 (263-275) sequence which are superior in their capacity to induce an immune response and in their tolerizing capacity.

It was surprisingly found that specific peptide modifications based on HC gp-39 (263-275) are agonistic for a set of T-cell hybridomas specific for HC gp-39 (263-275) peptide and superior in stimulation of two human T-cell clones generated following stimulation with peptides accommodating the 263-275 epitope sequence. Moreover, these modified peptides showed a superior tolerizing capacity *in vivo*.

Thus according to one aspect of the invention there is provided a modified peptide derived from H-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH (formula I; SEQ ID NO:1) having general formula Q- A1-A2-A3-A4-A5-A6-A7-A8-A9-A10-A11-A12-A13- Z (formula II). In general formula II, A1 through A13 correspond with the amino acids of formula I, Q corresponds with H and Z corresponds with OH. The modifications according to the present invention are selected from the group consisting of

a) substitution of 1-6, preferably 1-4 amino acids at A1 through A13 with non-natural amino acids or β amino acids

b) substitution of one or more amide bonds with reduced amide bonds or ethylene isosteres

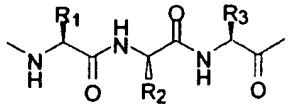
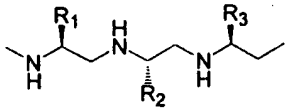
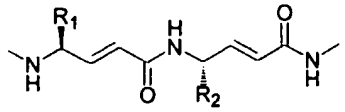
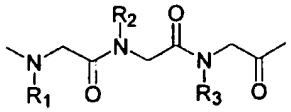
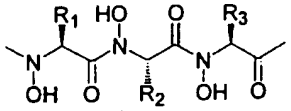
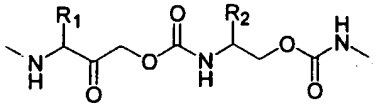
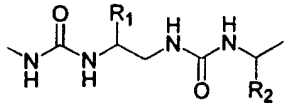
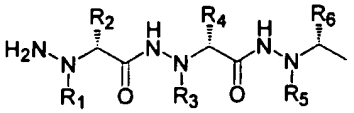
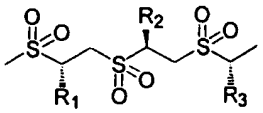
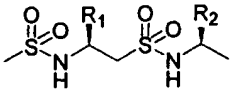
c) substitutions at Q and/or Z.

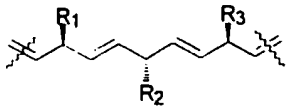
The number of modifications to be selected from one or more of these groups amounts 1-6. In addition the amino acids may be substituted with other natural amino acids provided that the total number of modifications does not exceed the number of 6.

Modified peptides based on formula I (HC gp-39 (263-275)) may be stabilised by C- and/or N- terminal modifications, which will decrease exopeptidase catalysed hydrolysis. Such modifications may include N-terminal acylation, (e.g. acetylation = Ac-peptide), C-terminal amide introduction, (e.g. peptide-NH_2), combinations of acylation and amide introduction (e.g. Ac-peptide-NH_2) and e.g. introduction of D-

amino acids instead of L-amino acids. Other modifications are focused on the prevention of hydrolysis by endopeptidases.

Table 1. Peptide linkages

Structure	Name
	Peptide
	Reduced peptide
	Vinylogous peptides
	Peptoid
	N-hydroxy-peptide
	Oligocarbamates
	Oligourea
	Hydrazinopeptides
	Oligosulphone
	Peptidosulphonamides



Ethylene isostere

Examples of these modifications are introduction of D-amino acids instead of L-amino acids, modified amino acids, cyclisation within the peptide, introduction of modified peptide bonds, e.g. reduced peptide bonds ψ[CH_2NH] and peptoids (N-alkylated glycine derivatives).

Other peptide analogues may be related to the peptides of formula I or general formula II but instead of the conventional -NH-C(O)- peptide bonds, the linkages shown in Table 1, or any combination thereof may be used instead of the individual -NH-C(O)- bonds. If the amino group at the N-terminus has been removed (e.g. A1 = desaminoarginine in formula II) Q in formula II corresponds to no atom.

Preferred peptides according to the invention are peptides wherein Q is H, (C_{1-6})alkyl, formyl, (C_{1-6})alkylcarbonyl, carboxy(C_{1-6})alkyl, (C_{1-6})alkyloxycarbonyl, (C_{2-6})alkenyloxycarbonyl, (C_{6-14})aryl(C_{1-6})alkyl; (C_{6-14})aryl(C_{1-4})alkyloxycarbonyl, $CH_3(OCH_2CH_2)_n$-OCH_2-C(O)- wherein n is 1-10, $HOCH_2$-$(CHOH)_m$-CH_2- wherein m is 3-4; 1-methyl-pyridinium-3-carbonyl, 1-methyl-pyridinium-4-carbonyl or Lys or Q is absent if A1 is H_2N-C(=NH)NH-$(CH_2)_n$-C(O)-wherein n is 2-5;

Z is OR wherein R is H, (C_{1-6})alkyl, (C_{2-6})alkenyl, (C_{6-14})aryl(C_{1-4})alkyl, (C_{6-14})(C_{4-13})heteroaryl(C_{1-6})alkyl or NR_1R_2 wherein R_1 and R_2 are independently selected from H, (C_{1-6})alkyl or (C_{6-14})aryl(C_{1-6})alkyl;

and, optionally, Q and Z contain in addition together up to 10 amino acids located next to position A1 and/or A13. Substitution at A1 through A13 with one or more other natural amino acids preferably is performed at no more than four, more preferably two positions.

In the peptides according to the present invention the following substitutions at general formula II are to be preferred:

Q is H, (C_{1-6})alkyl, formyl, (C_{1-6})alkylcarbonyl, carboxy(C_{1-6})alkyl, (C_{1-6})alkyloxy-carbonyl, (C_{2-6})alkenyloxycarbonyl, (C_{6-14})aryl(C_{1-6})alkyl; (C_{6-14})aryl(C_{1-4})alkyloxycarbonyl, $CH_3(OCH_2CH_2)_n$-OCH_2-C(O)- wherein n is 1-10, $HOCH_2$-

$(CHOH)_m$-CH_2- wherein m is 3-4; 1-methyl-pyridinium-3-carbonyl, 1-methyl-pyridinium-4-carbonyl or Lys, or Q is absent if A1 is H_2N-C(=NH)NH-$(CH_2)_n$-C(O)- wherein n is 2-5. More preferred are substitions wherein Q is H, (C_{1-6})alkyl, (C_{1-6})alkylcarbonyl, carboxy(C_{1-6})alkyl, (C_{1-6})alkyloxycarbonyl, $CH_3(OCH_2CH_2)_n$-OCH_2-C(O)- wherein n is 1-10, $HOCH_2$-$(CHOH)_m$-CH_2- wherein m is 3-4; 1-methyl-pyridinium-3-carbonyl, 1-methyl-pyridinium-4-carbonyl or Lys, or Q is absent if A1 is H_2N-C(=NH)NH-$(CH_2)_n$-C(O)-wherein n is 2-5. Even more preferred are peptides wherein is Q is H, methyl; acetyl; carboxymethylene, methoxycarbonyl; $CH_3(OCH_2CH_2)_3$-OCH_2-C(O)-, D-1-glucityl, 1-methyl-pyridinium-3-carbonyl or 1-methyl-pyridinium-4-carbonyl, or Q is absent if A1 is H_2N-C(=NH)NH-$(CH_2)_4$-C(O)-.

A1 is L-Arg, D-Arg, L-Lys, D-Lys, L-Ala, D-Ala, H_2N-C(=NH)NH-$(CH_2)_n$-C(O)- wherein n is 2-5, H_2N-$(CH_2)_n$-C(O)-, wherein n is 2-7, *(R)*-{-NH-CH[$(CH_2)_n$-NH-C(=NH)-NH_2]-CH_2-C(O)-}, wherein n is 2-5 or *(S)*-{-NH-CH[$(CH_2)_n$-NH-C(=NH)-NH_2]-CH_2-C(O)-}, wherein n is 2-5 or -N[$(CH_2)_n$-NH-C(=NH)-NH_2]CH_2C(O)-, wherein n is 2-5. Preferably A1 is L-Arg, D-Arg, L-Ala, H_2N-C(=NH)NH-$(CH_2)_n$-C(O)-wherein n is 2-5, H_2N-$(CH_2)_n$-C(O)-, wherein n is 2-7, *(S)*-{-NH-CH[$(CH_2)_n$-NH-C(=NH)-NH_2]-CH_2-C(O)-}, wherein n is 2-5 or -N[$(CH_2)_n$-NH-C(=NH)-NH_2]CH_2C(O)-, wherein n is 2-5. More preferably A1 is L-Arg, D-Arg, L-Ala, H_2N-C(=NH)NH-$(CH_2)_4$-C(O)-, H_2N-$(CH_2)_n$-C(O)-, wherein n is 5-7, *(S)*-{-NH-CH[$(CH_2)_3$-NH-C(=NH)-NH_2]-CH_2-C(O)-} or -N[$(CH_2)_3$-NH-C(=NH)-NH_2]CH_2C(O)-.

A2 is L-Ser, D-Ser, L-hSer, D-hSer, L-Thr, D-Thr, L-Ala, D-Ala, Gly or -N[$(CH_2)_n$-OH]-CH_2-C(O)- wherein n is 2-5. Preferably A2 is L-Ser, L-Ala, D-Ala, Gly or -N[$(CH_2)_n$-OH]-CH_2-C(O)- wherein n is 2-5. More preferably A2 is L-Ser, L-Ala or -N[$(CH_2)_2$-OH]-CH_2-C(O)-.

A3 is L-Phe, D-Phe, L-Phe(X), D-Phe(X) wherein X is independently selected from one or more of (C_{1-4})alkyl, hydroxy, halo, (C_{1-6})alkylcarbonylamino, amino or nitro, L-Hfe, D-Hfe, L-Thi, D-Thi, L-Cha, D-Cha, L-Pal(3), D-Pal(3), L-1-Nal, D-1-Nal, L-2-Nal, D-2-Nal, L-Ser(Bzl), D-Ser(Bzl), *(R)*-{-NH-CH(CH_2-aryl)-CH_2-} or *(S)*-{-NH-CH(CH_2-aryl)-CH_2-} or *(R)*-{-NH-CH(CH_2-aryl)-CH_2-} or *(S)*-{-NH-CH(CH_2-aryl)-CH_2-}. Preferably A3 is L-Phe, D-Phe, L-Phe(X) or D-Phe(X) wherein X is halo or nitro, L-Hfe, L-Thi, L-Cha, L-Pal(3), L-1-Nal, L-2-Nal, L-Ser(Bzl) or *(S)*-{-NH-

$CH(CH_2\text{-aryl})\text{-}CH_2\text{-}\}$. More preferably A3 is L-Phe, D-Phe, L-Phe(X) wherein X is halo or nitro, L-Hfe, L-Thi, L-Cha, L-Pal(3), L-1-Nal, L-2-Nal or L-Ser(Bzl).

A4 is L-Thr, D-Thr- L-Ser-, D-Ser, L-hSer, D-hSer, L-Ala, D-Ala or Gly. Preferably A4 is L-Thr or L-Ala.

A5 is L-Leu, D-Leu, L-Ile, D-Ile, L-Val, D-Val-, L-Nva, D-Nva, L-Ala, D-Ala, Gly, *(R)*-$\{\text{-NH-CH}(CH_2\text{-CH}(CH_3)_2)\text{-}CH_2\text{-}\}$, or *(S)*-$\{\text{-NH-CH}(CH_2\text{-CH}(CH_3)_2)\text{-}CH_2\text{-}\}$. Preferably A5 is L-Leu, L-Ala, or *(S)*-$\{\text{-NH-CH}(CH_2\text{-CH}(CH_3)_2)\text{-}CH_2\text{-}\}$.

A6 is L-Ala, D-Ala or Gly. Preferably A6 is L-Ala or Gly.

A7 is L-Ser, D-Ser, L-hSer, D-hSer, L-Thr, D-Thr, L-Ala, D-Ala or Gly. Preferably A7 is L-Ser or L-Ala.

A8 is L-Ser, D-Ser, L-hSer, D-hSer, L-Thr, D-Thr, L-Ala, D-Ala or Gly. Preferably A8 is L-Ser or L-Ala.

A9 is L-Glu, D-Glu, L-Asp, D-Asp, L-Ala, D-Ala or Gly. Preferably A9 is L-Glu or L-Ala.

A10 is L-Thr, D-Thr, L-Ser, D-Ser, L-hSer, D-hSer, L-Ala, D-Ala or Gly. Preferably A10 is L-Thr or L-Ala.

A11 is Gly, L-Ala, D-Ala or $\text{-NH-}CH_2\text{-}CH_2\text{-}$. Preferably A11 is Gly, L-Ala or $\text{-NH-}CH_2\text{-}CH_2\text{-}$.

A12 is L-Val, D-Val, L-Nva, D-Nva, L-Leu, D-Leu, L-Ile, D-Ile, *(R)*-$\{\text{-NH-CH}[CH(CH_3)_2]\text{-}CH_2\text{-}\}$, *(S)*-$\{\text{-NH-CH}[CH(CH_3)_2]\text{-}CH_2\text{-}\}$, *(R)*-$\{\text{-NH-CH}[CH_2CH_2CH_3]\text{-}CH_2\text{-}\}$, *(S)*-$\{\text{-NH-CH}[CH_2CH_2CH_3]\text{-}CH_2\text{-}\}$, *(R)*-$\{\text{-NH-CH}[CH_2CH(CH_3)_2]\text{-}CH_2\text{-}\}$, *(S)*-$\{\text{-NH-CH}[CH_2CH(CH_3)_2]\text{-}CH_2\text{-}\}$, *(RR)*-$\{\text{-NH-CH}[CH_2(CH(CH_3)\text{-}CH_2CH_3]\text{-}CH_2\text{-}\}$, *(RS)*-$\{\text{-NH-CH}[CH_2(CH(CH_3)\text{-}CH_2CH_3]\text{-}CH_2\text{-}\}$, *(SR)*-$\{\text{-NH-CH}[CH_2(CH(CH_3)\text{-}CH_2CH_3]\text{-}CH_2\text{-}$, or *(SS)*-$\{\text{-NH-CH}[CH_2(CH(CH_3)\text{-}CH_2CH_3]\text{-}CH_2\text{-}\}$. Preferably A12 is L-Val or *(S)*-$\{\text{-NH-CH}[CH(CH_3)_2]\text{-}CH_2\text{-}\}$.

A13 is Gly, L-Ala or D-Ala. Preferably A13 is Gly or L-Ala

Furthermore, in the peptides according to the invention Z is OR wherein R is H, $(C_{1\text{-}6})$alkyl, $(C_{2\text{-}6})$alkenyl, $(C_{6\text{-}14})$aryl$(C_{1\text{-}4})$alkyl, $(C_{4\text{-}13})$heteroaryl$(C_{1\text{-}6})$alkyl or NR_1R_2 wherein R_1 and R_2 are independently selected from H, $(C_{1\text{-}6})$alkyl or $(C_{6\text{-}14})$aryl$(C_{1\text{-}6})$alkyl. Preferably Z is OR wherein R is H or NR_1R_2 wherein R_1 and R_2 are independently selected from H or $(C_{1\text{-}6})$alkyl. More preferably Z is OH, NH_2 or NHEt.

The peptides according to the invention optionally may be extended at the N and C terminal end, i.e. next to A1 and /or A13 with several amino acids. Preferably they may be extended with up to 10 amino acids. Thus, Q and Z may contain in addition together up to 10 amino acids located next to position A1 and/or A13.

The peptides may differ from general formula I at several positions but preferably they are modified at 1-4 positions, more preferably at 2-3 positions.

As used herein the term (C_{1-6})alkyl means a branched or unbranched alkyl group having 1-6 carbon atoms, for example methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl and hexyl. Most preferred are alkyl groups having 1-4 carbon atoms.

The term (C_{1-4})alkyl means a branched or unbranched alkyl group having 1-4 carbon atoms.

The term (C_{2-6})alkenyl means a branched or unbranched alkenyl group having 2-6 carbon atoms, such as ethenyl, 2-butenyl etc. (C_{1-4})Alkenyl groups are preferred, (C_{1-3})alkenyl groups being the most preferred.

The term (C_{1-6})alkylcarbonyl means a branched or unbranched alkyl group having 1-6 carbon atoms, attached to a carbonyl group, for example an acetyl group. Most preferred are alkyl groups having 1-4 carbon atoms.

The term carboxy-(C_{1-6})alkyl means a carboxy group attached to a branched or unbranched alkyl group having 1-6 carbon atoms. Most preferred are alkyl groups having 1-4 carbon atoms.

The term (C_{1-6})alkyloxycarbonyl means a branched or unbranched alkyl group, attached to an oxycarbonyl group, for example a methoxycarbonyl-, or a *tert*-butyloxycarbonyl-(Boc-) group. Most preferred are alkyl groups having 1-4 carbon atoms.

The term (C_{2-6})alkenyloxycarbonyl means a branched or unbranched alkenyl group having 2-6 carbon atoms as defined previously, attached to an oxycarbonyl group, for example an allyloxycarbonyl group. (C_{1-4})Alkenyl groups are preferred, (C_{1-3})alkenyl groups being the most preferred.

The term (C_{1-6})(di)alkylamino means a (di)alkylamino group having 1-6 carbon atoms, the alkyl moiety having the same meaning as previously defined. Preferred are alkyl groups having 1-4 carbon atoms.

The term amino(C_{1-6})acyl means an acyl group having 1-6 carbon atoms, functionalized with an amino group. Preferred are acyl groups having 1-4 carbon atoms.

The term (C_{6-14})aryl means an aromatic hydrocarbon group having 6-14 carbon atoms, such as phenyl, naphthyl, tetrahydronaphthyl, indenyl, anthracyl, which may optionally be substituted at the ortho and/or meta position with one or more substituents such as - but not limited to- hydroxy, halogen, nitro, cyano, amino((C_{1-6})acyl) or (di)(C_{1-6})alkylamino, the acyl and alkyl moiety having the same meaning as previously defined. (C_{6-10})Aryl groups are preferred, phenyl being the most preferred.

The term (C_{4-13})heteroaryl(C_{1-6})alkyl means a substituted or unsubstituted aromatic group having 4-13 carbon atoms, preferably 4-9, at least including one heteroatom selected from N, O and/or S, connected to a branched or an unbranched alkyl group having 1-6 carbon atoms. The substituents on the heteroaryl group may be selected from the group of substituents listed for the aryl group. Nitrogen-containing heteroaryl groups may either be connected via a carbon or a nitrogen atom to the alkyl group. Of the alkyl groups, groups having 1-4 carbon atoms are preferred.

The term (C_{1-6})alkyl(C_{6-14})aryl means means a branched or unbranched alkyl group as defined previously, attached to an aryl group as defined previously. (C_{6-10})Aryl groups are preferred, phenyl being the most preferred. Of the alkyl groups, groups having 1-4 carbon atoms are preferred.

The term (C_{6-14})aryl(C_{1-6})alkyl means an arylalkyl group, wherein the alkyl group is a (C_{1-6})alkyl group and the aryl group is a (C_{6-14})aryl as defined previously, for example a benzyl- (Bzl) or a triphenylmethyl- (Trt) group. (C_{6-10})Aryl groups are preferred, phenyl being the most preferred. Of the alkyl groups, groups having 1-4 carbon atoms are preferred.

The term (C_{1-6})alkylcarbonylamino means an alkylcarbonylamino group, the alkyl group of which contains 1-6 carbon atoms and has the same meaning as previously defined. Alkyl groups having 1-4 carbon atoms are preferred.

The term (C_{6-14})aryl(C_{1-4})alkyloxycarbonyl means an (C_{6-14})aryl group connected to an alkyloxycarbonyl group, wherin the alkyl group is a (C_{1-4})alkyl group, and the aryl group is defined as previously, for example a benzyloxycarbonyl- (Z) or an Fluorenyl-methoxycarbonyl- (Fmoc) group. (C_{6-10})Aryl groups are preferred, phenyl being the most preferred.

The term halo means F, Cl, Br or I.

The naturally occurring amino acids are shown using their abbreviations (3-letter code) as follows: alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), serine (Ser), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), threonine (Thr), tryptophan (Trp), tyrosine (Tyr) and valine (Val). Of all the amino acids the stereochemistry is defined as L-.

A non-natural amino acid is an, optionally *N*α-substituted, α-amino acid having a chemical structure not identical to those of the natural amino acids. Non-natural amino acids are e.g. Phe(X), with X is a substituent situated at the para position of the phenyl ring of Phe, hSer (2-amino-4-hydroxybutanoic acid), norleucine (Nle, 2-aminohexanoic acid), norvaline (Nva, 2-aminopentanoic acid), L-Hfe (L-α-homophenylalanine), D-Hfe (D-α-homophenylalanine), L-Thi (β-thienyl-L-alanine), D-Thi (β-thienyl-D-alanine), L-Cha (β-cyclohexyl-L-alanine), D-Cha (β-cyclohexyl-D-alanine), L-Pal(3) (β-3-pyridyl-L-alanine), D-Pal(3) (β-3-pyridyl-D-alanine), L-1-Nal (β-1-naphthyl-L-alanine), D-1-Nal (β-1-naphthyl-D-alanine), L-2-Nal (β-2-naphthyl-L-alanine), D-2-Nal (β-2-naphthyl-D-alanine), L-Ser(Bzl) (O-benzyl-L-serine), D-Ser(Bzl) (O-benzyl-D-serine) and N-alkylglycine derivatives such as NVal (N-isopropylglycine, NArg (N-(3-guanidinopropyl)glycine) and NhSer (N-(2-hydroxyethyl)glycine). Included within this group of amino acids are also the naturally occurring amino acids, the stereochemistry of which is defined as D-.

It is to be understood that in a peptide incorporating a reduced amide bond the original carbonyl group of the amino acid has been replaced by a methylene group. In a peptide incorporating an ethylene isostere the original carboxamide function (-C(O)-NR-) has been replaced by an ethylene group (-CH=CR-).

The term ψ[CH_2NH] between two amino acid residues in a sequence means that the original amide bond (-C(O)-NH-) between those amino acid residues has been replaced by a reduced amide bond (-CH_2NH-).

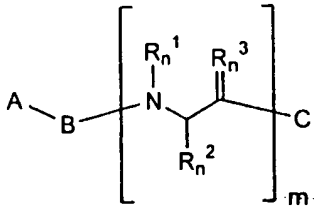
Several amino acids as indicated in formula I are preferred to be fixed at the corresponding positions at general formula 2. Thus, a preferred embodiment of the invention is a modified peptide having general formula Q-A1-A2-A3-Thr-Leu-Ala-Ser-Ser-Glu-Thr-A11-A12-Gly-Z (formula III) wherein Q, A1, A2, A3, A11, A12 and Z are as defined previously. The most preferred substitutions in general formula III are for A1

L-Arg, D-Arg, H_2N-C(=NH)NH-$(CH_2)_4$-C(O)-, H_2N-$(CH_2)_n$-C(O)-, wherein n is 5-7 or -N[$(CH_2)_3$-NH-C(=NH)-NH_2]CH_2C(O)-, for A2 L-Ser or -N[$(CH_2)_2$-OH]-CH_2-C(O)-,and for A3 L-Phe, L-Phe(X) wherein X is halo, L-1-Nal, L-2-Nal, L-Ser(Bzl), L-Thi, L-Cha or L-Pal(3).

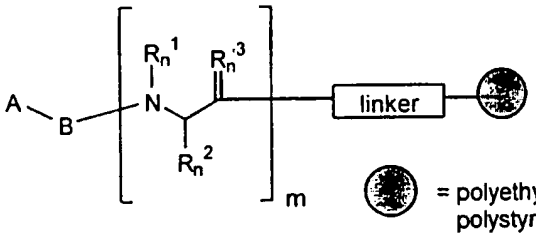
More preferred are peptides according to general formula III wherein A1 is Arg, A3 is Phe and A11 is Gly giving rise to general formula IV: Q-Arg-A2-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-A12-Gly-Z wherein the positions Q, A2, A12 and Z are as defined previously.

The most preferred peptides are selected from the group comprising desaminoargininyl-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2, desaminoargininyl-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, CH_3-$(OCH_2CH_2)_3$-OCH_2-C(O)-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2, D-1-glucityl-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, CH_3O-C(O)-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, Ac-Arg-Ser-Phe-ψ-[CH_2NH]-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2, Ac-Arg-Ser-Phe-Thr-Leu-ψ-[CH_2NH]-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2, Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ-[CH_2NH]-Gly-NH_2, Ac-Arg-N[$(CH_2)_2$-OH]-CH_2-C(O)-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly- NH_2, Ac-Arg-N[$(CH_2)_2$-OH]-CH_2-C(O)-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ-[CH_2NH]-Gly- NH_2, H-Arg-Ser-Phe(Cl)-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, H_2N-$(CH_2)_5$-C(O)-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, H_2N-$(CH_2)_6$-C(O)-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, (N-methyl-nicotinoyl)$^+$-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH.

A suitable methodology towards the synthesis of modified HC gp39 (263-275) peptides with *N*-terminal modifications, as visualized in Formula V, commences with derivatives of Formula VI. The modified peptides are synthesized *via* the commonly used Solid Phase Peptide Synthesis (SPPS) method (B. Merrifield, Solid Phase Peptide Synthesis, Peptides 1995, 93-169, Editor: B. Gutte, Academic, San Diego, California, USA; P. Lloyd-Williams, F. Albericio, E. Giralt, *Tetrahedron* 49: 11065-11133, 1993). Depending on the type of linker that is used, the peptide chain is connected to the support via either an ester (PAC linker) or an amide (PAL linker) linkage



Formula V



Formula VI

After anchoring of the Fmoc-*C*-terminal amino acid to the solid support (m = 1, A-B = Fmoc), using *e.g.* the coupling agents HATU (L. Carpino, A. El-Faham, C.A. Minor, F. Albericio, J. Chem. Soc., Chem. Comm. 201-203, 1994) or PyBOP (J. Coste, D. Le-Nguyen, B. Castro, Tetrahedron Lett. 31:205-208, 1990) and DiPEA, the chain is elongated (m = 2-12) by sequential acylation with the appropriately protected Fmoc-amino acid derivatives followed by piperidine-mediated removal of the Fmoc protective group (A-B = H) using an automated peptide synthesizer. Alternatively, pentafluorophenyl (Pfp) amino acid active esters (A. Dryland, R.C. Sheppard, Tetrahedron 44: 859-876, 1988) may be used to effect the condensations. Subsequently, the *N*-terminal amino acid B is introduced using the same protocol and the Fmoc-group is removed. The thus obtained 13-meric peptide derivative (A = H, B = *N*-terminal amino acid, m = 12) is then amenable to functionalization of the *N*-terminus. Introduction of an additional amide bond at the *N*-terminus (A = alkylcarbonyl) can be accomplished by performing another HATU or PyBOP-mediated condensation with the desired acid (X-OH) or by coupling with an acyl chloride (X-Cl) in the presence of pyridine. The charged 1-methyl pyridinium-4-carbonyl unit or 1-methyl pyridinium-3-carbonyl unit may be introduced after cleavage (*vide infra*) from the resin (*i.e* Formula V, A = H, B = *N*-terminal amino acid, Z = OR with R = H, alkyl or Z = NR^1R^2 with R^1, R^2 = H or alkyl) by DiPEA-mediated reaction of the free *N*-terminus of the completely deprotected peptide with the *N*-methyl (iso)nicotinium hydroxysuccinimide active ester (M.L. Tedjamulia, P.C. Srivastava, F.F. Knapp, J. Med. Chem., 28:1574-1580, 1985)

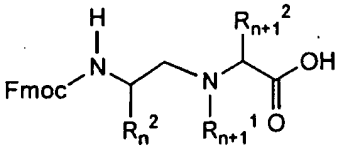
in aqueous medium (*cf.* conversion of A = H to A = *N*-methyl(iso)nicotinium in Formula V).

N-alkylation may be effected *via* reductive amination by treating the immobilized peptide with an appropriate aldehyde (Formula VI, conversion of A = H to A = alkyl) in the presence of $NaBH(OAc)_3$ in DMF/HOAc (99/1, v/v). Alternatively, reaction of the immobilized peptide (A = H) with an alkyl halide in the presence of DiPEA also gives access to *N*-alkylated peptides (*e.g.* reaction with *tert*-butyl bromoacetate). The free NH_2 (A = H in Formula VI) may also be functionalized with a carbamoyl group (*e.g* methoxycarbonyl) *via* reaction with the corresponding carbamoyl chloride in CH_2Cl_2/DiPEA (conversion of A = H to A = alkyloxycarbonyl in Formula VI). After cleavage of the peptide from the solid support with concomitant removal of the acid-labile protective groups using TFA/Et_3SiH/anisole/ROH (R = H, alkyl) the peptides with general Formula V (Z = OR) are purified by RP-HPLC. Alternatively, the *C*-terminus may be equipped with an amide function (Z = NR^1R^2 with R^1 and R^2 = H or alkyl) during cleavage from the resin. In that case, a different linker (PAL: B. Merrifield, Peptides, 93-169, 1995) between the peptide chain (Formula VI) and the PEG-PS solid support is used. If the free amino group of the PAL linker is alkylated prior to attachment of the first (*C*-terminal) amino acid, *C*-terminal alkyl amides will be formed after cleavage from the polymer support.

Using the same Fmoc-SPPS strategy, peptides are accessible that contain unnatural but commercially available amino acids (*e.g.* D-amino acids or substituted phenylalanine derivatives). Apart from this, *N*-alkyl glycine derivatives (peptoid monomers, R_n^1 = amino acid side chain, R_n^2 = H in Formulas V and VI) are first synthesized in solution using literature procedures (J.A. Kruijtzer, L.J.F. Hofmeyer, W. Heerma, C. Versluis, R.M.J. Liskamp, Chem. Eur. J. 4:1570-1580, 1998). Also the modified *N*-terminal amino acid β-homo-L-arginine [B = NH-CH($CH_2CH_2CH_2$NH-CH(=NH)NH_2)-CH_2-C(O)] was prepared in solution prior to SPPS, according to known procedures (H.M.M. Bastiaans, A.E. Alewijnse, J.L. van der Baan, H.C.J. Ottenheijm, Tetrahedron Lett. 35:7659-7660, 1994. After protection of the free NH_2 group in the thus obtained monomeric amino acids with a Fmoc protective group, the compounds can be incorporated in the elongating peptide (Formula VI) using the SPPS protocol.

The final class of modified peptides comprises peptides containing one or more reduced amide bonds (R_n^3 = H_2 in Formula V). These derivatives are accessible (J.J. Wen, A.R. Spatola, J. Pept. Res., 49:3-14, 1997) via a modified SPPS protocol in

which the free *N*-terminal amino acid of the growing chain (1<m<12 in Formula VI) is alkylated with the incoming amino acid aldehyde under reductive conditions ($NaBH_3CN$, DMF/HOAc, 99/1, v/v). The required *N*-Fmoc protected amino acid aldehydes are either commercially available or accessible by literature methods (J.J. Wen, C.M. Crews, Tetrahedron: Asymmetry 9:1855-1858, 1998). The thus elongated chain (A-B = Fmoc, R_n^1 = H, R_n^2 = amino acid side chain, R_n^3 = H_2) contains a secondary amino function (R_{n+1}^1 = H) which is subsequently protected with a Boc group. After removal of the Fmoc protective group the peptide chain may be further elongated using the SPPS protocol.



Formula VII

Alternatively, a dimeric structure with general Formula VII may be synthesized in solution prior to SPPS. Thus, the appropriate amino acid benzyl ester (H_2N-$CH(R_{n+1}^2)$-CO_2Bzl) is reductively alkylated ($NaBH_3CN$, DMF/HOAc, 99/1, v/v) with a Fmoc protected amino acid aldehyde (Fmoc-NH-$CH(R_n^2)$-C(O)H) to give a dimeric secondary amine. After protection of the amino function with a Boc group (R_{n+1}^1 = Boc) and subsequent hydrogenolysis of the benzyl ester a compound of Formula VII is formed, which can be incorporated into the growing chain using the SPPS procedure.

The peptides according to the invention can be used as a therapeutical substance. More particularly, they can be used for the induction of specific T-cell tolerance to an autoantigen in patients who are suffering from autoimmune disease disorders, more specifically arthritis.

Modified peptides based on a MHC class II restricted T-cell epitope structure with enhanced stimulatory activity *in vitro* and an enhanced activity *in vivo* can be selected using known technologies.

In order to maintain the agonistic properties of a given T-cell epitope it is regarded essential not to interfere too much with either the residues involved in binding to the

relevant MHC molecule nor influence too much the residues involved in TCR engagement of relevant T-cells. Thus, selection of agonistic modified peptides would involve:

1) definition of the affinity of a modified peptide for binding the relevant MHC molecule and comparison with the affinity of the wild type, the non-modified, peptide epitope

2) definition of the stimulatory activity of a modified peptide and comparison with the activity of the wild type, non-modified, peptide, using an *in vitro* assay (irradiated antigen presenting cells co-incubated with peptide antigen and specific T-cells). Preferably a broad panel of epitope-specific, MHC class II restricted T-cells, with different TCR clonotypes, but reactive with the same epitope in the context of the same MHC class II molecule, should be evaluated. For this purpose, a panel of specific T-cell hybridomas or specific T-cell lines/clones can be employed. Selection of a modified epitope for human application will preferably require the use of human T-cell lines/clones to safeguard the relevance of the selected modified epitopes for human T-cell recognition.

3) definition of the activity of a modified peptide *in vivo* (optional). For this purpose different experimental set-ups may be used

a) a delayed type hypersensitivity test

b) an *ex vivo* T-cell activation assay following the administration of antigen (with or without adjuvant) *in vivo*

c) modulation of disease in experimental models of autoimmune disease by administration of modified peptide antigen

Preferably compounds with enhanced agonistic activity *in vitro*, as compared to the wild type peptide or enhanced *in vivo* effects are to be selected.

Individual HC gp-39 derived peptides that are being recognised in mice are expected to downmodulate reactivity towards these peptides following nasal treatment. Such reactivity can be measured by challenging the animal with the peptide in question and quantitating paw swelling as a result of a DTH response. Peptide immunisations in Balb/c mice result in immunological responses to the HC gp-39 peptide 263-275. Thus, mice immunised with HC gp-39 can be challenged with HC gp-39 263-275 in order to detect a DTH response. To delineate tolerogenicity of modified peptides *in vivo*, mice

can be treated per nostril with HC gp-39 263-275 or peptide derivatives in various concentrations. Modified peptide derivatives with a superior profile in terms of tolerance induction are expected to be active in this *in vivo* assay in lower concentrations than the original peptide. To be able to quantitatively detect effects of tolerance induction with the native peptide versus modified peptide derivatives, various application schemes and dosages can be tested. Finally, it can be investigated whether modified forms of HC gp-39 263-275 are more effective in downmodulating HC gp-39 263-275 induced DTH responses in this model than the native 263-275 peptide.

Tolerance can be attained by administering high or low doses of the tolerogen or peptides according to the invention. The amount of tolerogen or peptide will depend on the route of administration, the time of administration, the age of the patient as well as general health conditions and diet.

In general, a dosage of 0.01 to 1000 μg of peptide or protein per kg body weight, preferably 0.05 to 500 μg, more preferably 0.1 to 100 μg of peptide or protein can be used.

Another aspect of the invention resides in pharmaceutical compositions comprising one or more of the peptides according to the invention and a pharmaceutical acceptable carrier.

Pharmaceutical acceptable carriers are well known to those skilled in the art and include, for example, sterile salin, lactose, sucrose, calcium phosphate, gelatin, dextrin, agar, pectin, peanut oil, olive oil, sesame oil and water. Other carriers may be, for example MHC class II molecules, if desired embedded in liposomes.

In addition the pharmaceutical composition according to the invention may comprise one or more adjuvants. Suitable adjuvants include, amongst others, aluminium hydroxide, aluminium phosphate, amphigen, tocophenols, monophosphenyl lipid A, muramyl dipeptide and saponins such as Quill A. The amount of adjuvant depends on the nature of the adjuvant itself.

Furthermore the pharmaceutical composition according to the invention may comprise one or more stabilizers such as, for example, carbohydrates including sorbitol, mannitol, starch, sucrosedextrin and glucose, proteins such as albumin or casein, and buffers like alkaline phosphates.

Suitable administration routes are intramuscular injections, subcutaneous injections, intravenous injections or intraperitoneal injections, oral and intranasal administration. Oral and intranasal administration are preferred administration routes. Especially, modulator cells specific for the antigen could be generated by applying the antigen via the mucosae, for instance the nasal mucosae. Mucosal administration of antigens has been shown to induce immunological tolerance to such antigens.

The peptides according to the invention are also very suitable for use in a diagnostic method to detect the presence of activated autoreactive T cells involved in the chronic inflammation of the articular cartilage.

The diagnostic method according to the invention comprises the following steps:

a) isolation of the peripheral blood mononuclear cells (PBMC) from a blood sample of an individual,

b) culture said PBMC under suitable conditions,

c) incubation of said PBMC culture in the presence of the autoantigen or one or more peptides derived thereof according to the invention, and

d) detection of a response of T cells, for example a proliferative response, indicating the presence of activated autoreactive T cells in the individual.

In case of detection of a response by measuring the proliferative response of the autoreactive T cells, the incorporation of a radioisotope such as for example 3H-thymidine is a measure for the proliferation. A response of the autoreactive T cells present in the PBMC can also be detected by measuring the cytokine release with cytokine-specific ELISA, or the cytotoxicity with ^{51}Chromium release. Another detection method is the measurement of expression of activation markers by FACS analysis, for example of Il-2R. A diagnostic composition comprising one or more of the peptides according to the invention and a suitable detecting agent thus forms part of the invention. Depending on the type of dection, the detection agent can be a radioisotope, an enzyme, or antibodies specific for cell surface or activation markers.

Also within the scope of the invention are test kits which comprise one or more peptides according to the invention. These test kits are suitable for use in a diagnostic method according to the invention.

Thus, according to the present invention HC gp-39 derived modified peptides can be used to downmodulate autoimmune disease.

The following examples are illustrative for the invention and should in no way be interpreted as limiting the scope of the invention.

Legends to the figures

Figure 1

Proliferation of clone 235 following stimulation with lead peptide or selected modified peptides using irradiated, autologous PBMC as APCs was measured as described in example 15. Peptides were tested for their stimulatory activity in concentrations of 0, 0.4, 2, 10 and 50 µg/ml. The response of the 235 clone following stimulation with lead peptide H-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH (closed circles), stimulation with Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ[CH_2NH]-Gly-NH_2 (closed squares), stimulation with Ac-Arg-NhSer-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH2 (open circles) or stimulation with Ac-Arg-NhSer-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ[CH_2NH]-Gly-NH_2 (open squares) is shown.

Table 2

Hybridoma assay (first line t est) : + = compound stimulates all three hybridomas in a fashion comparable to or superior to the non-modified 263-275 peptide. +* = agonist activity demonstrated for 1 or 2 hybridomas but not for all three. Reactivity of human clones (proliferation of clone 235 and 243) in potency (stimulatory activity of analogue/stimulatory activity of lead peptide; e.g.HC gp-39 (263-275)). - = potency < 0.6, + = potency 0.6 -12, ++ = potency >12 - 100, +++ = potency >100. Ac-Arg-NhSer-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2, Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ[CH_2NH]-Gly-NH_2, D-1-Glucityl-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, $CH_3(OCH_2CH_2)_3$-$OCH_2C(O)$-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2 and H-Arg-Ser-Phe(4Cl)-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH were tested for their affinity to bind HLA-DRB1*0401 and compared to the affinity of the lead-peptide (H-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH). The most active compounds (Ac-Arg-NhSer-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2 and Ac-Arg-Ser-Phe-Thr-Leu-

Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ[CH_2NH]-Gly-NH_2) showed a relative affinity for binding to HLA-DRB1*0401 which was comparable to the affinity of the original peptide.

Examples

Example 1

H-Arg-Ser-Phe(4Cl)-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH (1)

The reaction vessel of the Millipore 9050 PepSynthesizer was charged with 0.5 g of Fmoc-Gly-PAC-PEG-PS (commercially available at PerSeptive Biosystems, 0.20 mmol/g) resin, pre-swollen in N-methyl-pyrrolidinone (NMP). Removal of the Fmoc group in each coupling cycle was effected with piperidine/DMF (1:4 v/v). The coupling efficiencies were determined by spectroscopic analysis of the Fmoc-cleavage after each elongation step. In each coupling step 4 equivalents of the appropriate acid-labile side-chain-protected Fmoc amino acid were used. The double syringe mode of the synthesizer was used in which one syringe contains 0.50 M HATU in DMF p.a. and the other syringe contains 1.0 M DIPEA in DMF p.a. The main wash contained N-methyl-pyrrolidinone with 0.1% HOBt. The Analog Synthesis protocol was used. After removal of the final Fmoc group the resin with the immobilized peptide was taken out of the reaction vessel and washed successively with DMF (20 mL), CH_2Cl_2 (20 mL), diethylether (20 mL), CH_2Cl_2 (20 mL), diethylether (20 mL), CH_2Cl_2 (20 mL) and diethylether (20 mL). The immobilized peptide was dried in vacuo overnight. The peptide was then cleaved off with 10 mL of the mixture TFA/$(iPr)_3SiH$/anisole/H_2O 88/5/5/2 v/v/v/v for 3 hours. In this step all the acid-labile side-chain protective groups were also removed. After evaporation of the solvent the peptide was precipitated with 200 mL of diethyl ether. The ether layer was decanted and the peptide was washed with an additional amount (2 x 200 mL) of ether. The crude peptide was then dried with a stream of nitrogen and lyophilized. Purification of the peptide was carried out by HPLC chromatography on a PrepPak cartridge 40-100 mm Delta-Pak™ C18 15 µm 100A reverse phase column. The mobile phase consisted of a mixture of 20% of phosphate buffer pH 2.1 and a gradient of acetonitrile and water, as shown in the analysis below. The peptide was desalted on the HPLC, using 4‰ aqueous acetic acid. The purified product was lyophilized.

Mobile phase: A: 0.5 mol/L NaH_2PO_4 + H_3PO_4, pH=2.1

B: H_2O

C: CH_3CN/H_2O = 3/2 (v/v)

gradient: A: 20%; B: 80% → 20%; C: 0% → 60% in 40 min.

Yield: 68 mg; HPLC purity: 90.1%; MS: MW = 1346, this agrees with the molecular formula $C_{55}H_{89}ClN_{16}O_{21}$; amino acid analysis: all amino acids were found in the required amounts; peptide content: 74.8%; ion chromatography: phosphate: 0.6%, acetate: 0.6%, chloride: 3.4% (w/w).

Example 2

H_2N-$(CH_2)_5$-C(O)-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH (2)

The peptide was synthesized using solid phase peptide chemistry, as reported in the synthesis of compound **1** (see above). In this case, commercially available Fmoc-amino acid pentafluorophenyl (Pfp) active esters were used instead of the free Fmoc-amino acids and HATU/DIPEA. The compound was prepared using 6-Fmoc-amino-hexanoic acid as the N-terminal amino acid, obtained from 6-amino-hexanoic acid, analogous to the literature procedure (A. Marston, E. Hecker, Z. Naturforsch. B Anorg. Chem. Org. Chem., 38:1015-1021,1983). The support was Fmoc-Gly-PAC-PEG-PS (0.75 g, 0.170 mmol/g) and 3 equiv. Of the appropriate Pfp esters were used. For the coupling of 6-Fmoc-amino-hexanoic acid PyBOP was applied as the coupling agent (199 mg). Workup as reported in the standard procedure (example 1) gave 168 mg of crude product. This was purified by HPLC (phosphate system pH=2.1, with CH_3CN-H_2O gradient). The product was desalted on the HPLC with 5 ‰ aqueous acetic acid and freeze-dried to give 34 mg of the required peptide.

HPLC purity: 99.6%; MS: MW = 1268, this agrees with the molecular formula $C_{55}H_{89}N_{13}O_{21}$; amino acid analysis: all amino acids were found in the required amounts; peptide content: 67.3%; ion chromatography: phosphate: 10% (w/w).

Example 3

H_2N-$(CH_2)_6$-C(O)-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH (3)

Peptide **3** was prepared in an identical fashion as its N-terminal homolog **2** using 7-Fmoc-amino-heptanoic acid (**3a**, prepared analogous to compound **2a**: A. Marston, E. Hecker, Z. Naturforsch. B Anorg. Chem. Org. Chem., 38:1015-1021, 1983) as the N-terminal amino acid. The support was Fmoc-Gly-PAC-PEG-PS (1.0 g, 0.17 mmol/g). Workup, HPLC purification and desalting as reported in the standard procedure (example 1) gave 45 mg of the required peptide.

HPLC purity: 95.0%; MS: MW = 1282, this agrees with the molecular formula $C_{56}H_{91}N_{13}O_{21}$; amino acid analysis: all amino acids were found in the required amounts; peptide content: 87.4%; ion chromatography: acetate: 0.2% (w/w).

Example 4

(N-methyl-nicotinoyl)$^+$-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH (4)

Prior to the preparation of peptide **4**, the starting material N-succinimidyl (1-methyl-3-pyridinio)formate iodide (**4a**) was synthesized via a literature procedure (M.L. Tedjamulia, P.C. Srivastava, F.F. Knapp; J. Med. Chem. 28:1574-1580, 1985). The synthesis of compond **4** was carried out in solution. The peptide H-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH (**4b**, 26 mg, 0.02 mmol), prepared by SPPS methods according to example 1, was dissolved in DMF/H_2O (1/99 v/v, 10 mL) and DIPEA/DMF (1/1, v/v) was added to give pH=9. Then N-succinimidyl (1-methyl-3-pyridinio)formate iodide (**4a**, 0.056 g. 0.15 mmol) was added in two portions. The pH was kept at pH=9 by adding a few drops of DIPEA/DMF (1/1, v/v). The mixture was stirred at room temperature for 4 hours and then diluted with 10 mL of H_2O and 5 mL of phosphate buffer pH=2.1. The product was purified immediately by HPLC with the phosphate buffer system as shown previously (example 1). Desalting with 5‰ aqueous acetic acid and lyophilisation provided 14 mg of the required peptide **4**.

HPLC purity: 98.1%; MS: MW = 1430; amino acid analysis: all amino acids were found in the required amounts; peptide content: 56.3%; ion chromatography: chloride: 1.4%, phosphate: 1.0%, trifluoroacetate: 0.8%, acetate: 0.3% (w/w).

Example 5

Desaminoargininyl-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH (5)

Peptide **5** was synthesized according to the previously described procedure for compound **1** using Fmoc-protected amino acids, HATU, DIPEA and 1.0 g of Fmoc-Gly-PAC-PEG-PS-resin, support loading 0.17 mmol/g. In the final step desamino-Arg(Adoc)$_2$-OH (**5a**) was coupled to the immobilized peptide chain. Carboxylic acid **5a** was prepared according to a known procedure (R. Presentini, G. Antoni, Int. J. Pept. Protein Res., 27:123-126, 1986). Workup and purification conditions were identical to those of peptide **1**.

Yield: 58 mg; HPLC purity: 91.1%; MS: MW = 1296; amino acid analysis: all amino acids were found in the required amounts; peptide content: 76.2%; ion chromatography: phosphate: 0.4%, trifluoroacetate: 0.6%, acetate: 0.2% (w/w).

Example 6

Desaminoargininyl-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH$_2$ (6)

The assembly of peptide **6** was conducted in a fashion similar to that of previously described peptide **5**, using PAL-PEG-PS resin (0.17 mmol/g) instead of PAC-PEG-PS as the solid support. In this case, the Fmoc group from commercially available (PerSeptive Biosystems) Fmoc-PAL-PEG-PS resin was removed and the resulting H-PAL-PEG-PS support was condensed with Fmoc-Gly-OH under the agency of HATU/DIPEA. After elongation of the peptide chain and subsequent cleavage from the resin under the same conditions as described in example 1, the required carboxamide C-terminus was obtained. Workup and purification conditions were identical to those of peptide **1**.

Yield: 43 mg; HPLC purity: 91.3%; MS: MW = 1295; amino acid analysis: all amino acids were found in the required amounts; peptide content: 76.5%; ion chromatography: chloride: 0.5%, acetate: 4.0% (w/w).

Example 7

CH$_3$(OCH$_2$CH$_2$)$_3$-OCH$_2$-C(O)-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH$_2$ (7)

For the synthesis of peptide **7** the starting material CH$_3$(OCH$_2$CH$_2$)$_3$-OCH$_2$-CO$_2$H (**7a**) was prepared first, according to a literature procedure (A.H. Haines, P. Karntiang,

Carbohydr. Res., 78:205-211, 1980). The synthesis of the protected and immobilized peptide H-Arg(Pmc)-Ser(tBu)-Phe-Thr(tBu)-Leu-Ala-Ser(tBu)-Ser(tBu)-Glu(OtBu)-Thr(tBu)-Gly-Val-Gly-PAL-PEG-PS (**7b**) was conducted as shown in example 2 using amino acid Pfp esters. The peptide on the resin (**7b**) was pre-swollen in NMP and 142 mg (0.64 mmol) of $CH_3(OCH_2CH_2)_3$-OCH_2CO_2H (**7a**) was added, together with 169 mg (0.64 mmol) of the coupling agent TFFH (tetramethylfluoro-formamidinium hexafluorophosphate). The combined reagents were circulated during 60 minutes in de Pepsynthesizer. Cleavage from the resin and workup were executed as described in example 5. The crude peptide was then purified by HPLC with the system and solvents delineated in example 1. The product was desalted on the HPLC column using 2.5‰ of AcOH.

Yield: 120 mg; HPLC purity: 78%; MS: MW = 1515; ion chromatography: chloride: 0.1%, phosphate: 0.3%, trifluoroacetate: 4.0%, acetate: 0.3% (w/w).

Example 8

D-1-glucityl-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH (8)

Prior to the assembly of N-terminally modified peptide **8**, the peptide H-Arg(Pmc)-Ser(tBu)-Phe-Thr(tBu)-Leu-Ala-Ser(tBu)-Ser(tBu)-Glu(OtBu)-Thr(tBu)-Gly-Val-Gly-PAC-PEG-PS (**8a**), having the same sequence as peptide **7b** but differing in the type of linker (PAC instead of PAL), was prepared according to example 1. Reductive amination was effected by overnight treatment of 6-*O*-trityl-α/β-D-glucopyranose (**8b**, 422 mg, 1.0 mmol, T. Utamura, K. Kuromatsu, K. Suwa, K. Koizumi, T. Shingu, Tetsuro; Chem. Pharm. Bull. 34:2341-2353, 1986) with the immobilized peptide **8a** (500 mg, 0.2 mmol/g) in DMF/HOAc (99/1, v/v, 10 mL) using $NaBH(Oac)_3$ (212 mg, 1.0 mmol) as the reducing agent. Subsequent cleavage of the resulting fully protected derivative (6-*O*-trityl-D-1-glucityl)-Arg(Pmc)-Ser(tBu)-Phe-Thr(tBu)-Leu-Ala-Ser(tBu)-Ser(tBu)-Glu(OtBu)-Thr(tBu)-Gly-Val-Gly-PAC-PEG-PS from the resin using the conditions described in example 1, with concomitant removal of the trityl group and all amino-acid-protecting groups, furnished 38 mg of the target peptide **8**, after purification by preparative HPLC and desalting with 5 ‰ aqueous HOAc.

HPLC purity: 84.7%; MS: MW = 1475; amino acid analysis: all amino acids were found in the required amounts; peptide content: 61.0%; ion chromatography: chloride: 0.1%, acetate: 1.7% (w/w).

Example 9

MeO-C(O)-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH (9)

The synthesis of peptide **9** commenced by suspending the immobilized peptide H-Arg(Pmc)-Ser(tBu)-Phe-Thr(tBu)-Leu-Ala-Ser(tBu)-Ser(tBu)-Glu(OtBu)-Thr(tBu)-Gly-Val-Gly-PAC-PEG-PS (**8a**) in dioxane and cooling to 0 ^{0}C. To this suspension, 100 µl of 4N aq NaOH and 100 µl of methyl chloroformate were added. The reaction mixture was agitated for 16 h and subsequently, the resin was washed with EtOH/H_2O, EtOH, CH_2Cl_2 and ether. After drying in vacuo, the product was cleaved off the resin and purified as described in the previous peptide syntheses (example 1). Finally, the peptide was desalted on the HPLC using 5‰ of aqueous acetic acid and then lyophilized to give peptide **9**.

Yield: 11 mg; HPLC purity: 96.8%; MS: MW = 1368; amino acid analysis: all amino acids were found in the required amounts; peptide content: 60.5%; ion chromatography: chloride: 2.0%, phosphate: 0.2%, acetate: 0.4% (w/w).

Example 10

Ac-Arg-Ser-Phe-Thr-Leu-ψ[CH_2NH]-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2 (10)

Prior to the synthesis of peptide **10**, the required amino acid aldehyde building block Fmoc-Leu-H (**10a**) was prepared via a known procedure (J.-P. Meyer, P. Davis, K.B. Lee, F. Porreca, H.I. Yamamura, V. Hruby, J. Med. Chem. 38:3462-3468, 1995). Compound **10a** was used without further purification. According to the method described in example 1, the resin was functionalized with an 8-amino acid peptide chain to give H-Ala-Ser(tBu)-Ser(tBu)-Glu(OtBu)-Thr(tBu)-Gly-Val-Gly-PAL-PEG-PS (**10b**). The latter immobilized derivative (1 g, 0.2 mmol/g) was suspended in 5 mL of 1% acetic acid in DMF. Two solutions were prepared, being 148 mg of Fmoc-Leu-H (**10a**) in 2.5 mL of DMF and 30 mg of $NaCNBH_3$ in 2.5 mL of DMF. Both solutions were combined and added to the suspension of peptide **10b**. The mixture was agitated overnight at room temperature. Subsequently, the thus obtained intermediate Fmoc-Leu-ψ[CH_2NH]-Ala-Ser(tBu)-Ser(tBu)-Glu(OtBu)-Thr(tBu)-Gly-Val-Gly-PAL-PEG-PS (**10c**) was protected at the newly introduced secondary amine function with Boc_2O and pyridine. The resin-bound peptide **10c** was suspended in 10 mL of dry CH_2Cl_2 and 35 mg (0.16 mmol) of Boc_2O and 13 µl (0.16 mmol) of pyridine were added. The pH

was kept at pH=8 with pyridine and the mixture was agitated overnight. Workup involved washing of the resin with CH_2Cl_2, EtOH, CH_2Cl_2, ether and drying in vacuo. The synthesis was continued by SPPS using Fmoc amino acids and the HATU/DIPEA protocol with NMP as the solvent (example 1). The last step involved coupling with 4-nitrophenyl acetate to introduce the N-terminal acetyl group. After workup as described in example 1, the crude peptide was purified by HPLC, desalted with 5 $^0/_{00}$ of acetic acid and freeze-dried to give the target peptide **10**.

Yield: 28 mg; HPLC purity: 76.3%; MS: MW = 1339; ion chromatography: trifluoroacetate: 1.2%, acetate: 2.0% (w/w).

Example 11

Ac-Arg-Ser-Phe-ψ[CH_2NH]-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2 (11)

The synthesis of **11** involved the reductive coupling of Fmoc-Phe-H (**11a**, J.-P. Meyer, P. Davis, K.B. Lee, F. Porreca, H.I. Yamamura, V. Hruby, J. Med. Chem., 38:3462-3468, 1995) to the resin-bound protected peptide H-Thr(tBu)-Leu-Ala-Ser(tBu)-Ser(tBu)-Glu(OtBu)-Thr(tBu)-Gly-Val-Gly-PAL-PEG-PS (**11b**) obtained via the SPPS protocol described in example 1. Peptide **11b** (1.0 g, 0.2 mmol/g) and aldehyde **11a** (200 mg) were suspended in 5 mL of 1% acetic acid/DMF and immediately 30 mg (0.48 mmol) of $NaCNBH_3$, dissolved in 5 mL of DMF, was added. The mixture was stirred for 16 h, resulting in the formation of Fmoc-Phe-ψ[CH_2NH]-Thr(tBu)-Leu-Ala-Ser(tBu)-Ser(tBu)-Glu(OtBu)-Thr(tBu)-Gly-Val-Gly-PAL-PEG-PS. The peptide chain was then elongated with the appropriate Fmoc-amino acids and N-terminal acetylating agent using the HATU/DIPEA SPPS protocol as described in example 8. Workup, HPLC-purification and desalting were carried out as described in example 1.

Yield: 52 mg; HPLC purity: 97.9%; MS: MW = 1338; amino acid analysis: all amino acids were found in the required amounts; peptide content: 92.4%; ion chromatography: acetate: 2.5% (w/w).

Example 12

Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ[CH_2NH]-Gly-NH_2 (12)

For the synthesis of this compound, it was impossible to carry out the reductive alkylation with Fmoc-Val-H on the resin. Therefore, the dipeptide analogue Fmoc-Val-

ψ[CH_2NH]-Gly-OH (**12d**) was prepared in solution prior to immobilization to the resin.

Fmoc-Val-ψ[CH_2NH]-Gly-Obzl (12c)

Fmoc-Val-H (**12a**, 3.16 g, 10 mmol, prepared according to T. Moriwake, S.-I. Hamano, S. Saito, S. Torii, S. Kashino, J. Org. Chem., 54:4114-4120, 1989) was dissolved in EtOH/HOAc (80 mL, 99/1, v/v) and HCl.H-Gly-Obzl (**12b**, 2.02 g, 10 mmol) was added, followed by $NaCNBH_3$ (0.94 g, 15 mmol). The reaction mixture was stirred at room temperature overnight. Subsequently, 5% aq. $NaHCO_3$ (20 mL) was added to neutralize the reaction mixture. The mixture was then concentrated in vacuo and the residue was extracted with CH_2Cl_2. The combined organic layers were washed with satd. Aq. NaCl, dried quickly over Na_2SO_4, filtered and the solvent was evaporated to yield a yellow oil. After purification by silica gel chromatography (eluent: 0-4% methanol in CH_2Cl_2) compound **12c** was isolated as a white solid. Yield: 1.85 g (39 %). Analysis: TLC: (silica, CH_2Cl_2/MeOH 98/2) Rf = 0.45, MS: MW = 472.

Fmoc-Val-ψ[CH_2N(Boc)]-Gly-Obzl (12d)

Fmoc-Val-ψ[CH_2NH]-Gly-Obzl (**12c**, 0.910 g, 1.93 mmol), Boc_2O (0.420 g, 1.93 mmol) and DIPEA (0.336 g, 1.93 mmol) were dissolved in dry CH_2Cl_2 (20 mL). The pH was kept basic by addition of DIPEA and the mixture was stirred overnight at room temperature. The reaction mixture was then acidified by addition of 10% $KHSO_4$. Water was added and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with aq. Satd. NaCl, dried quickly over $MgSO_4$ and the solvent was evaporated to yield 0.96 g (97%) of **12d**. Analysis: TLC: (silica, CH_2Cl_2/MeOH 98/2) Rf = 0.55; MS: MW=572.

Fmoc-Val-ψ[CH_2N(Boc)]-Gly-OH (12e)

Fmoc-Val-ψ[CH_2N(Boc)]-Gly-Obzl (**12d**, 0.97 g, 1.70 mmol) was dissolved in a mixture of MeOH/EtOAc (1/1, v/v, 100 mL) and hydrogenated at normal pressure with 10% Pd/C for a time of 2 h. The palladium catalyst was filtered off and the filtrate was concentrated to afford carboxylic acid **12e** as a slightly yellow oil. Yield: 0.661 g

(81%). Analysis: TLC (silica, CH_2Cl_2/MeOH/AcOH 90/9/1) Rf = 0.42; MS: MW = 482.

Using the Peptide synthesizer with HATU/DIPEA double syringe mode and with a double coupling with HATU/DIPEA, the compound Fmoc-Val-ψ[CH_2N(Boc)]-Gly-OH (**12e**) (0.661 g, 1.37 mmol) was loaded onto PAL-PEG-PS resin (1.5 g, 0.15 mmol/g, 0.225 mmol). The substitution level was measured with the standard Fmoc cleavage procedure and was 0.13 mmol/g of loaded resin (yield: 87%). The resulting peptide H-Val-ψ[CH_2N(Boc)]-Gly-PAL-PEG-PS (**12f**) was further elongated using the HATU/DIPEA SPPS protocol (example 1) with double condensation steps of 60 min for each Fmoc-amino acid. Similar to peptides **9** and **11** the N-terminal acetyl group was introduced using 4-nitrophenyl acetate. Workup, purification and desalting were carried out as described in example 1.

Yield: 17 mg; HPLC purity: 80.1%; MS: MW = 1338; amino acid analysis: all amino acids were found in the required amounts; peptide content: 63.7%; ion chromatography: chloride: 1.0%, phosphate: 0.2%, acetate: 0.2% (w/w).

Example 13

Ac-Arg-NhSer-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2 (13)

For the synthesis of peptide **13** the requisite peptoid monomer Fmoc-NhSer(tBu)-OH **(13e)** was prepared first.

Z-2-aminoethyl-*tert*-butyl ether (13a)

3.25 g $MgSO_4$ (27 mmol) was suspended in 80 mL of CH_2Cl_2 (dry). Under N_2 1.5 mL of conc. H_2SO_4 was added (procedure: S.W. Wright, D.L. Hageman, A.S. Wright, L. McClure, Tetrahedron Lett., 38:7345-7348, 1997). The mixture was stirred for 15 min after which *tert*-BuOH (12.9 mL) and commercially available Z-2-aminoethanol (5.28 g, 27 mmol), dissolved in CH_2Cl_2 (20 mL), were added. After stirring for 5 days, 200 mL of 5% aq. $NaHCO_3$ was added to the reaction mixture, which was stirred until all the $MgSO_4$ had dissolved. The layers were separated and the CH_2Cl_2 layer was washed with brine. The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated to yield 5.6 g of crude **13a**. The product was purified by column

chromatography (eluent: heptane/EtOAc 3:1 v/v). Yield 5.00 g (78%). ^{1}H NMR ($CDCl_3$) δ: 1.15 (s, 9H, tBu), 3.3-3.5 (dt, 4H, 2 x CH_2), 5.1 (bs, 2H, CH_2Bzl), 7.4 (m, 5H, Ar).

2-aminoethyl-*tert*-butyl ether.HCl (13b)

To a solution of 5.00 g of benzyl ester **13a** in ethyl acetate (150 mL) 225 mg of 10% Pd/C was added and H_2 was bubbled through for 2 hours. The catalyst was filtered off and 15 mL of 1 M aq. HCl was added. The solvent was evaporated and a small volume of ether was added. The precipitated product **13b** was filtered off and dried in vacuo. Yield: 2.35 g (77%). NMR ($CDCl_3$) δ: 1.20 (s, 9H, tBu), 3.15 (t, 2H, CH_2), 3.65 (t, 2H CH_2), 8.1-8.4 (bs, 2H, NH_2)

N-(2-*tert*-butoxyethyl)-glycine (H-NhSer(tBu)-OH) (13c)

To a solution of **13b** (2.30 g, 15 mmol) in 25 mL of H_2O was added 1.40 g (15.2 mmol) of glyoxylic acid.H_2O. The pH was adjusted to pH=6 with 1.0 M aq NaOH. To this solution 230 mg of Pd/C was added and the reaction mixture was agitated at 45 psi H_2 pressure overnight. The catalyst was filtered off and washed with 5 mL H_2O. The filtrate containing **13c** was used without further purification in the next step.

Fmoc-NhSer(tBu)-OH (13d)

The reaction product **13c**, still dissolved in H_2O, was brought to pH=9.5 with 1 N NaOH. The basic solution was diluted with 25 mL of acetone and 5.40 g (16 mmol) Fmoc-Osu, dissolved in 25 mL of acetone, was added dropwise. The pH was kept at pH=9.5 with 1 N NaOH. After stirring overnight, the reaction mixture was concentrated to 150 mL and washed with 2 x 50 mL of ether/heptane (1/1, v/v). The H_2O layer was acidified to pH = 2.5 with 20% citric acid and 3 x extracted with 100 mL of ethyl acetate. The organic layers were combined and dried over Na_2SO_4. The solvent was evaporated and the product was purified by column chromatography (silica, CH_2Cl_2/MeOH 5/1, v/v) and freeze-dried. Yield: 5.44 g (91 %). ^{1}H NMR ($CDCl_3$) δ: 1.20 (s, 9H, tBu), 3.2 (dt, 2H CH_2), 3.6-3.7, (dt, 2H, CH_2), 4.05 (s, 2H, CH_2CO_2H), 4.2 (b, 1H Fmoc), 4.4-4.6 (2H, 2 x d, Fmoc), 7.3-7.8 (m, 8H, ArH, Fmoc).

The synthesis of peptide **13** was carried out on the Pepsynthesizer using the dual syringe technique as described before (example 1). The support was Fmoc-PAL-PEG-PS, (1.0 g, 0.15 mmol/g) with NMP as the solvent. Double couplings (coupling time 60 min) were used for all amino acids, including Fmoc-NhSer(tBu)-OH **(13d)**. The N-terminal acetyl group was introduced using 4-nitrophenyl acetate. Workup and cleaving off the resin and protecting groups were conducted in the standard way (example 1). The crude peptide was purified by HPLC and desalted with 5‰ of aqueous acetic acid.

Yield: 50 mg; HPLC purity: 98.6%; MS: MW = 1366; amino acid analysis: all amino acids were found in the required amounts; peptide content: 82.1%; ion chromatography: chloride: 0.3%, acetate: 1.3% (w/w).

Example 14

Ac-Arg-NhSer-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ[CH_2NH]-Gly-NH_2 (14)

The synthesis was carried out using the HATU/DIPEA protocol on the Pepsynthesizer (example 1). The previously described functionalized resin H-Val-ψ[CH_2N(Boc)]-Gly-PAL-PEG-PS **(12f)** and the protected peptoid Fmoc-NhSer(tBu)-OH **(13d)** were used as building blocks. As described before, the dual syringe technique and double couplings of 60 min per coupling were used. Elongation of the peptide chain on the synthesizer was stopped before the coupling of Fmoc-NhSer(tBu)-OH **(13d)** and this amino acid was dissolved in DMSO with sonication prior to coupling to the immobilized peptide chain (H-Phe-Thr(tBu)-Leu-Ala-Ser(tBu)-Ser(tBu)-Glu(OtBu)-Thr(tBu)-Gly-Val-ψ[CH_2NH]-Gly-PAL-PEG-PS). The synthesis was finished by condensation of the remaining (Arg) amino acid and acetylation using 4-nitrophenyl acetate. Workup, purification and desalting of the peptide were standard, as outlined in example 1. Lyophilization furnished 47 mg of peptide **14**.

HPLC purity: 72.9%; MS: MW = 1352; ion chromatography: trifluoroacetate: 5.5% (w/w).

Example 15

Pre-selection of agonist peptides using antigen-specific T-cell hybridomas (first line test).

To test the agonist activity of a modified peptide, 3 different, HC gp-39 (263-275)-specific hybridoma cell lines were used (5G11, 8B12 and 14G11). 5 x 10^4 hybridoma cells and 2 x 10^5 irradiated (12000 RAD), EBV-transformed B cells carrying the DRB1*0401 specificity were incubated in 150 µl volumes in wells of a round-bottomed microtiter plate. Peptide antigen (HC gp-39 (263-275), and modified peptides) was added in 50 µl volumes to duplicate wells. Forty eight hr later 100 µl of the culture supernatant was assayed for antigen-specific IL-2 production using a sandwich ELISA with Pharmingen antibodies specific for mouse IL-2.

Selection of agonist peptides using antigen-specific T-cell clones (second line test)

The 243 T-cell clone was isolated from a peptide-specific T-cell line obtained from an RA responder to peptide 263-275 (RA patient 243). The clones were obtained following four repetitive stimulations with HC gp-39 (263-275) peptide in the presence of DRB1*0401-matched PBMC. The H235 T-cell clone was isolated from a peptide-stimulated T-cell line obtained from an HLA-DRB1*0401-positive donor. Upon 2 stimulations with peptide HC gp-39 (261-275) in the presence of DRB1*0401-matched PBMC, clones were obtained by PHA cloning. Both clone 243 and 235 were found to be HLA-DRB1*0401 restricted in the recognition of peptide antigen. Cells were used on day 10-14 after stimulation in each experiment.

Proliferative responses of clone 243 or clone 235 were measured by incubation of 2 x 10^4 T cells and 10^5 DRB1*0401-matched (3,000 Rad irradiated) PBMC in 150 µl volumes of medium with 10% normal human pool serum (NHS, CLB, Amsterdam, The Netherlands) in flat-bottomed microtiter plates. 50 µl of antigen solution (containing the 263-275 sequence or modifications as indicated) was distributed in triplicate wells. ^{3}H-thymidine was added at day 2 or 3 of incubation. Cells were harvested on glass fibre filters and the incorporated radioactivity was measured.

Results

Most modified peptides as listed in Table 2 were able to stimulate all three T-cell hybridomas in a fashion comparable to the lead peptide H-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH. Some peptides, however, did not stimulate all three hybridomas which exemplifies the difference in specificity of the hybridomas used.

When these agonists were tested for their capacity to stimulate the two human T-cell clones, a clear difference in potency of the compounds tested became obvious (Table 2). Most modified compounds induced a response of clone 235 and clone 243. One compound (Ac-Narg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH2) did not induce a proliferative response of either clone. Three compounds (H-betahomoargininyl-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, Ac-Arg-Ser-Phe-ψ[CH2NH]-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2 and Ac-Arg-Ser-Phe-Thr-Leu-ψ[CH2NH]-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2) were active on one clone only (either clone 243 or 235). Three compounds (H-Arg-Ser-Phe(4Cl)-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, H-D-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH and $CH_3OC(O)$-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH) induced a proliferative response in both clones which was in the same order of magnitude as the response induced by the lead peptide H-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH. Seven compounds (Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, $CH_3(OCH_2CH_2)_3$-$OCH_2C(O)$-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2, D-1-glucityl-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, (N-methyl-nicotinoyl)$^+$-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ[CH_2NH]-Gly-NH_2, Ac-Arg-NhSer-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2 and Ac-Arg-NhSer-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ[CH_2NH]-Gly-NH_2) were superior in inducing a proliferative response of one or both clones. The most potent compounds identified being Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ[CH_2NH]-Gly-NH_2, Ac-Arg-NhSer-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2 and Ac-Arg-NhSer-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ[CH_2NH]-Gly-NH_2 (Table 2 and Figure 1).

Example 16

Female Balb/c mice of approximately 8-10 weeks of age (Charles River Germany or Charles River France) were immunised on day 0 with 100 µl of antigen preparation (50 µg of HC gp-39 263-275) in Incomplete Freunds Adjuvant (IFA; Sigma Chemicals, St. Louis, USA). Antigen was given subcutaneously in two portions in the chest region of the mice. On day 7, mice were challenged with antigen preparation (HC gp-39 (263-275)) diluted in 0.9% NaCl (NPBI, Emmer Compascuum, The Netherlands) in a volume of 50 µl in 1 mg/ml alum (Pharmacy Donkers-Peterse, Oss, The Netherlands)

unilaterally in the footpad (left paw); the other (right) footpad was injected with 50 µl of alum solution in 0.9% NaCl as a control. Delayed type hypersensitivity responses (mean % specific swelling) were determined on day 8 by measuring the increase in footpad thickness of the left hind footpad compared to the right hind footpad (swelling left (mm) - swelling right (mm) / swelling right (mm) x 100%), using a in-house designed micrometer.

Nasal application of antigen preparation (50, 10, 2 or 0.4 µg (or lower concentrations)) of HC gp-39 (263-275) or of modified peptide derivatives was performed under Isoflurane (Forene®, Abbott BV, Amstelveen, The Netherlands) anesthesia once (day -5) before immunisation on day 0 with 100 µl of antigen preparation containing 50 µg of HC gp-39 263-275 in IFA. In these experiments, mice were immunised and challenged with HC gp-39 263-275 and DTH responses were determined as described above.

Using the above described assay system in which Balb/c mice immunised with HC gp-39 (263-275) in IFA responded to HC gp-39 (263-275), it became possible to study the potential effects of tolerance induction by nasal application of HC gp-39 (263-275) in comparison to those of modified peptide derivatives. Pre-treatment with HC gp-39 (263-275) downmodulated the HC gp-39 (263-275) specific DTH reaction; this effect was dependent on the dose of peptide that was included in the pre-treatment procedure. Using a relatively high peptide concentration (50 µg/mouse), nasal application of one dose of HC gp-39 (263-275) fully abrogated the DTH reaction whereas a dose of 2 µg/mouse was ineffective. Thus, a protocol was established to discriminate between effective (tolerogenic) and ineffective doses of peptide in a HC gp-39 (263-275) specific DTH assay system. Assuming that modified peptide derivatives based on HC gp-39 (263-275) may be active at lower concentrations than the original peptide, such peptides are expected to induce tolerance at relatively low peptide concentrations. Following this assumption, a series of modified peptides was tested in this tolerance induction protocol. In this experiment (in which a reliable HC gp-39 (263-275) response was induced that could be downmodulated by pre-treatment with 50 but not 2 µg of HC gp-39 (263-275)) it was shown that specific modifications of the peptide were highly active in the induction of tolerance whereas others were not (see Table 3).

Table 2 Summary of results obtained in the first line (hybridoma assay) and second line (human clone proliferation assay) tests

Peptides tested	Hybridoma assay	Reactivity of human clones	
		235	243
H-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	+	+	+
H-Arg-Ser-Phe(4Cl)-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	+	+	+
H-Arg-Ser-Phe(4Br)-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	+	nd	nd
H-Arg-Ser-Cha-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	+	nd	nd
Ac-(D-Arg)-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	+	+	+
Desaminoargininyl-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2	+	nd	nd
Desaminoargininyl-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	+	nd	nd
Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	+	+++	+++
CH_3-$(OCH_2CH_2)_3$-$OCH_2C(O)$-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2	+	+	++
D-Glucityl-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	+	++	++
(N-Me-nicotininoyl)$^+$-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	+	+	++
MeO-C(O)-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	+	+	+
H-betahomoargininyl-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	+	+	-
Ac-Arg-Ser-Phe-ψ[CH_2NH]-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2	+*	-	nd
Ac-Arg-Ser-Phe-Thr-Leu-ψ[CH_2NH]-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2	+*	+	-
Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-ψ[CH_2NH]-Val-Gly-NH_2	+*	nd	nd
Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ[CH_2NH]-Gly-NH_2	+	+++	+++
Ac-Narg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2	+	-	-
Ac-Arg-NhSer-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2	+	+++	+++
Ac-Arg-NhSer-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ[CH_2NH]-Gly-NH_2	+	+++	+++

Table 3 DTH tests

Peptides tested	Dose tested (DTH) (μg i.n day -5)	Half-tolerogenic dose (μg) 1)
H-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	50, 10, 2, 0.4	10
H-Arg-Ser-Phe(4Cl)-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	50, 10, 2	<2
H-Arg-Ser-Phe(4Br)-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	50, 10, 2, 0.4	2-10
H-Arg-Ser-Cha-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	50, 10, 2	50
Ac-(D-Arg)-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	50, 10, 2, 0.4	-
Desaminoargininyl-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH2	50, 10, 2	50-10
Desaminoargininyl-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	50, 10, 2, 0.4	<0.4
Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	50, 10, 2, 0.4	-
CH3-(OCH2CH2)3-OCH2C(O)-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH2	50, 10, 2,	<2
D-Glucityl-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	50, 10, 2, 0.4	<0.4
(N-Me-nicotininoyl)+-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	50, 10, 2, 0.4	10-50
MeO-C(O)-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	50, 10, 2, 0.4	2-10
H-betahomoargininyl-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH	ND	
Ac-Arg-Ser-Phe-ψ[CH2NH]-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH2	50, 10, 2, 0.4	<0.4
Ac-Arg-Ser-Phe-Thr-Leu-ψ[CH2NH]-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH2	50, 10, 2	10-50
Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-ψ[CH2NH]-Val-Gly-NH2	ND	
Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ[CH2NH]-Gly-NH2	50, 10, 2, 0.4, 0.08, 0.016, 0.0032	0.08
Ac-Narg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH2	ND	
Ac-Arg-NhSer-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH2	50, 10, 2, 0.4, 0.08, 0.016, 0.0032	0.016-0.08
Ac-Arg-NhSer-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ[CH2NH]-Gly-NH2	50, 10, 2, 0.4, 0.08, 0.016, 0.0032	0.0032

1) half tolerogenic dose: tested dose with 50% inhibition of DTH respons (max DTH in that particular experiment). -: not tolerogenic ND: not done.

Claims

1. A modified peptide derived from H-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH (formula I) having general formula Q- A1-A2-A3-A4-A5-A6-A7-A8-A9-A10-A11-A12-A13- Z (formula II) wherein A1 through A13 correspond with the amino acids of formula I, Q corresponds with H and Z corresponds with OH characterized in that 1 to 6 modifications are selected from one or more of the groups a, b or c consisting of
a) substitution of 1-6, preferably 1-4 amino acids at A1 through A13 with non-natural amino acids or β amino acids
b) substitution of one or more amide bonds with reduced amide bonds or ethylene isosteres
c) substitution at Q and/or Z; and, optionally,
d) substitution with natural amino acids up to a total of 6 modifications.

2. The peptide according to claim 1 wherein
Q is H, (C_{1-6})alkyl, formyl, (C_{1-6})alkylcarbonyl, carboxy(C_{1-6})alkyl, (C_{1-6})alkyl-oxycarbonyl, (C_{2-6})alkenyloxycarbonyl, (C_{6-14})aryl(C_{1-6})alkyl; (C_{6-14})aryl(C_{1-4})alkyloxycarbonyl, $CH_3(OCH_2CH_2)_n$-OCH_2-C(O)- wherein n is 1-10, $HOCH_2$-$(CHOH)_m$-CH_2- wherein m is 3-4; 1-methyl-pyridinium-3-carbonyl, 1-methyl-pyridinium-4-carbonyl or Lys, or Q is absent if A1 is H_2N-C(=NH)NH-$(CH_2)_n$-C(O)-wherein n is 2-5;
Z is OR wherein R is H, (C_{1-6})alkyl, (C_{2-6})alkenyl, aryl(C_{1-4})alkyl, (C_{4-13})heteroaryl(C_{1-6})alkyl or NR_1R_2 wherein R_1 and R_2 are independently selected from H, (C_{1-6})alkyl or (C_{6-14})aryl(C_{1-6})alkyl;
and, optionally, Q and Z comprise in addition together up to 10 amino acids located next to position A1 and/or A13.

3. The peptide according to claims 1 or 2 wherein the substitutions with the natural amino acids at A1 through A13 occur at no more than 4, preferably no more than 2 positions.

4. The peptide according to claims 1-3 wherein
Q is H, (C_{1-6})alkyl, formyl, (C_{1-6})alkylcarbonyl, carboxy(C_{1-6})alkyl, (C_{1-6})alkyl-oxycarbonyl, (C_{2-6})alkenyloxycarbonyl, aryl(C_{1-6})alkyl; (C_{6-14})aryl(C_{1-4})alkyloxycarbonyl, $CH_3(OCH_2CH_2)_n$-OCH_2-C(O)- wherein n is 1-10, $HOCH_2$-$(CHOH)_m$-CH_2- wherein m is 3-4; 1-methyl-pyridinium-3-carbonyl, 1-methyl-pyridinium-4-carbonyl or Lys, or Q is absent if A1 is H_2N-C(=NH)NH-$(CH_2)_n$-

C(O)-wherein n is 2-5;

A1 is L-Arg, D-Arg, L-Lys, D-Lys, L-Ala, D-Ala, H_2N-C(=NH)NH-$(CH_2)_n$-C(O)-wherein n is 2-5, H_2N-$(CH_2)_n$-C(O)-, wherein n is 2-7, *(R)*-{-NH-CH[$(CH_2)_n$-NH-C(=NH)-NH_2]-CH_2-C(O)-}, wherein n is 2-5 or *(S)*-{-NH-CH[$(CH_2)_n$-NH-C(=NH)-NH_2]-CH_2-C(O)-}, wherein n is 2-5 or -N[$(CH_2)_n$-NH-C(=NH)-NH_2]CH_2C(O)-, wherein n is 2-5;

A2 is L-Ser, D-Ser, L-hSer, D-hSer, L-Thr, D-Thr, L-Ala, D-Ala, Gly or -N[$(CH_2)_n$-OH]-CH_2-C(O)- wherein n is 2-5;

A3 is L-Phe, D-Phe, L-Phe(X), D-Phe(X) wherein X is independently selected from one or more of (C_{1-4})alkyl, hydroxy, halo, (C_{1-6})alkylcarbonylamino, amino or nitro, L-Hfe, D-Hfe, L-Thi, D-Thi, L-Cha, D-Cha, L-Pal(3), D-Pal(3), L-1-Nal, D-1-Nal, L-2-Nal, D-2-Nal, L-Ser(Bzl), D-Ser(Bzl), *(R)*-{-NH-CH(CH_2-aryl)-CH_2-} or *(S)*-{-NH-CH(CH_2-aryl)-CH_2-} or *(R)*-{-NH-CH(CH_2-aryl)-CH_2-} or *(S)*-{-NH-CH(CH_2-aryl)-CH_2-};

A4 is L-Thr, D-Thr- L-Ser-, D-Ser, L-hSer, D-hSer, L-Ala, D-Ala or Gly;

A5 is L-Leu, D-Leu, L-Ile, D-Ile, L-Val, D-Val-, L-Nva, D-Nva, L-Ala, D-Ala, Gly, *(R)*-{-NH-CH(CH_2-$CH(CH_3)_2$)-CH_2-}, or *(S)*-{-NH-CH(CH_2-$CH(CH_3)_2$)-CH_2-};

A6 is L-Ala, D-Ala or Gly;

A7 is L-Ser, D-Ser, L-hSer, D-hSer, L-Thr, D-Thr, L-Ala, D-Ala or Gly;

A8 is L-Ser, D-Ser, L-hSer, D-hSer, L-Thr, D-Thr, L-Ala, D-Ala or Gly;

A9 is L-Glu, D-Glu, L-Asp, D-Asp, L-Ala, D-Ala or Gly;

A10 is L-Thr, D-Thr, L-Ser, D-Ser, L-hSer, D-hSer, L-Ala, D-Ala or Gly;

A11 is Gly, L-Ala, D-Ala or -NH-CH_2-CH_2-;

A12 is L-Val, D-Val, L-Nva, D-Nva, L-Leu, D-Leu, L-Ile, D-Ile, *(R)*-{-NH-CH[$CH(CH_3)_2$]-CH_2-}, *(S)*-{-NH-CH[$CH(CH_3)_2$]-CH_2-}, *(R)*-{-NH-CH[$CH_2CH_2CH_3$]-CH_2-}, *(S)*-{-NH-CH[$CH_2CH_2CH_3$]-CH_2-}, *(R)*-{-NH-CH[$CH_2CH(CH_3)_2$]-CH_2-}, *(S)*-{-NH-CH[$CH_2CH(CH_3)_2$]-CH_2-}, *(RR)*-{-NH-CH[CH_2($CH(CH_3)$-CH_2CH_3]-CH_2-}, *(RS)*-{-NH-CH[CH_2($CH(CH_3)$-CH_2CH_3]-CH_2-}, *(SR)*-{-NH-CH[CH_2($CH(CH_3)$-CH_2CH_3]-CH_2-, or *(SS)*-{-NH-CH[CH_2($CH(CH_3)$-CH_2CH_3]-CH_2-};

A13 is Gly, L-Ala or D-Ala and

Z is OR wherein R is H, (C_{1-6})alkyl, (C_{2-6})alkenyl, (C_{6-14})aryl(C_{1-4})alkyl, (C_{4-13})heteroaryl(C_{1-6})alkyl or NR_1R_2 wherein R_1 and R_2 are independently selected from H, (C_{1-6})alkyl or (C_{6-14})aryl(C_{1-6})alkyl and, optionally,

Q and Z contain in addition together up to 10 amino acids located next to position A1 and/or A13.

5. The peptide according to claims 1-4 wherein

Q is H, (C_{1-6})alkyl, (C_{1-6})alkylcarbonyl, carboxy(C_{1-6})alkyl, (C_{1-6})alkyloxy-carbonyl, $CH_3(OCH_2CH_2)_n$-OCH_2-C(O)- wherein n is 1-10, $HOCH_2$-$(CHOH)_m$-CH_2- wherein m is 3-4; 1-methyl-pyridinium-3-carbonyl, 1-methyl-pyridinium-4-carbonyl or Lys, or Q is absent if A1 is H_2N-C(=NH)NH-$(CH_2)_n$-C(O)-wherein n is 2-5;

A1 is L-Arg, D-Arg, L-Ala, H_2N-C(=NH)NH-$(CH_2)_n$-C(O)-wherein n is 2-5, H_2N-$(CH_2)_n$-C(O)-, wherein n is 2-7, *(S)*-{-NH-CH[$(CH_2)_n$-NH-C(=NH)-NH_2]-CH_2-C(O)-}, wherein n is 2-5 or -N[$(CH_2)_n$-NH-C(=NH)-NH_2]CH_2C(O)-, wherein n is 2-5;

A2 is L-Ser, L-Ala, D-Ala, Gly or -N[$(CH_2)_n$-OH]-CH_2-C(O)- wherein n is 2-5;

A3 is L-Phe, D-Phe, L-Phe(X) or D-Phe(X) wherein X is halo or nitro, L-Hfe, L-Thi, L-Cha, L-Pal(3), L-1-Nal, L-2-Nal, L-Ser(Bzl) or *(S)*-{-NH-CH(CH_2-aryl)-CH_2-};

A4 is L-Thr or L-Ala;

A5 is L-Leu, L-Ala, or *(S)*-{-NH-CH(CH_2-CH(CH_3)$_2$)-CH_2-};

A6 is L-Ala or Gly;

A7 is L-Ser or L-Ala;

A8 is L-Ser or L-Ala;

A9 is L-Glu or L-Ala;

A10 is L-Thr or L-Ala;

A11 is Gly, L-Ala or -NH-CH_2-CH_2-;

A12 is L-Val or *(S)*-{-NH-CH[$CH(CH_3)_2$]-CH_2-};

A13 is Gly or L-Ala and

Z is OR wherein R is H or NR_1R_2 wherein R_1 and R_2 are independently selected from H or (C_{1-6})alkyl and, optionally,

Q and Z contain in addition together up to 10 amino acids located next to position A1 and/or A13.

6. The peptide according to claims 1-5 wherein

Q is H, methyl; acetyl; carboxymethylene, methoxycarbonyl; $CH_3(OCH_2CH_2)_3$-OCH_2-C(O)-, D-1-glucityl, 1-methyl-pyridinium-3-carbonyl or 1-methyl-pyridinium-4-carbonyl, or Q is absent if A1 is H_2N-C(=NH)NH-$(CH_2)_4$-C(O)-;

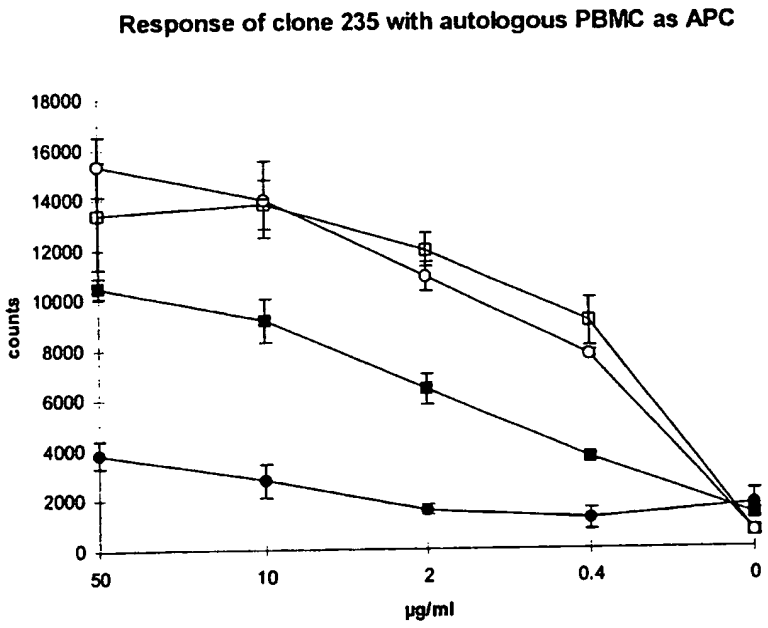
A1 is L-Arg, D-Arg, L-Ala, H_2N-C(=NH)NH-$(CH_2)_4$-C(O)-, H_2N-$(CH_2)_n$-C(O)-, wherein n is 5-7, *(S)*-{-NH-CH[$(CH_2)_3$-NH-C(=NH)-NH_2]-CH_2-C(O)-} or -

$N[(CH_2)_3$-NH-C(=NH)-$NH_2]CH_2C(O)$-;
A2 is L-Ser, L-Ala or -$N[(CH_2)_2$-OH]-CH_2-C(O)-;
A3 is L-Phe, D-Phe, L-Phe(X) wherein X is halo or nitro, L-Hfe, L-Thi, L-Cha, L-Pal(3), L-1-Nal, L-2-Nal or L-Ser(Bzl) and
Z is OH, NH_2 or NHEt and, optionally,
Q and Z contain in addition together up to 10 amino acids located next to position A1 and/or A13.

7. The peptide according to claims 1-6 wherein the general formula is Q-A1-A2-A3-Thr-Leu-Ala-Ser-Ser-Glu-Thr-A11-A12-Gly-Z (formula III)
8. The peptide according to claims 1-7 having 1-4 modifications.
9. The peptide according claim 8 having 2-3 modifications.
10. The peptide according to claim 7 wherein
A1 is L-Arg, D-Arg, H_2N-C(=NH)NH-$(CH_2)_4$-C(O)-, H_2N-$(CH_2)_n$-C(O)-, wherein n is 5-7 or -$N[(CH_2)_3$-NH-C(=NH)-$NH_2]CH_2C(O)$-,
A2 is L-Ser or -$N[(CH_2)_2$-OH]-CH_2-C(O)-,
A3 is L-Phe, L-Phe(X) wherein X is halo, L-1-Nal, L-2-Nal, L-Ser(Bzl), L-Thi, L-Cha or L-Pal(3).
11. The peptide according to claims 9 or 10 wherein the general formula is Q-Arg-A2-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-A12-Gly-Z (formula IV).
12. A peptide selected from the group comprising desaminoargininyl-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2, desaminoargininyl-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, CH_3-$(OCH_2CH_2)_3$-OCH_2-C(O)-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2, D-1-glucityl-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, CH_3O-C(O)-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, Ac-Arg-Ser-Phe-ψ-$[CH_2NH]$-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2, Ac-Arg-Ser-Phe-Thr-Leu-ψ-$[CH_2NH]$-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-NH_2, Ac-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ-$[CH_2NH]$-Gly-NH_2, Ac-Arg-$N[(CH_2)_2$-OH]-CH_2-C(O)-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly- NH_2, Ac-Arg-$N[(CH_2)_2$-OH]-CH_2-C(O)-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-ψ-$[CH_2NH]$-Gly- NH_2, H-Arg-Ser-Phe(Cl)-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, H_2N-$(CH_2)_5$-C(O)-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, H_2N-$(CH_2)_6$-C(O)-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH, (N-methyl-nicotinoyl)$^+$-Arg-Ser-Phe-Thr-Leu-Ala-Ser-Ser-Glu-Thr-Gly-Val-Gly-OH.

13. A peptide according to any of the claims 1-12 for use as a therapeutical substance.
14. Pharmaceutical composition comprising one or more of the peptides according to claims 1-12, and a pharmaceutically acceptable carrier.
15. Use of one or more of the peptides according to claims 1-12 for the manufacture of a pharmaceutical preparation for the induction of specific T-cell tolerance to an autoantigen in patients suffering from autoimmune disorders, more specifically arthritis.
16. Diagnostic composition comprising one or more of the peptides according to any of the claims 1-12 and a detection agent.

Figure 1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µg/ml

SEQUENCE LISTING

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<120> Modified peptides and peptidomimetics for use in immunotherapy

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<170> PatentIn Ver. 2.1

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<220>
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<220>
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<210> 9
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<223> Description of Artificial Sequence: Synthetic peptide

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<223> Description of Artificial Sequence: Synthetic peptide

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INTERNATIONAL SEARCH REPORT

Inter ›nal Application No
PCT/EP 00/10230

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/47 G01N33/68 A61K38/10 A61P19/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 40068 A (BOOTS ANNA MARIA HELENA ;AKZO NOBEL NV (NL); VERHEIJDEN GIJSBERTUS) 30 October 1997 (1997-10-30) abstract page 21, line 8 - line 9 page 25, line 17 Seq Id No 41 ---	1,2, 4-11, 13-16
X	EP 0 856 520 A (AKZO NOBEL NV) 5 August 1998 (1998-08-05) Seq Id Nos 2,3 abstract --- -/--	1,2,4-11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance

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'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 February 2001	09/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Ceder, O

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 00/10230

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 97 46578 A (EDWARDS PHILIP NEIL ;LUKE RICHARD WILLIAM ARTHUR (GB); COTTON RONA) 11 December 1997 (1997-12-11) the whole document	1-16
A	WO 96 13517 A (AKZO NOBEL NV ;BOOTS ANNA MARIA HELENA (NL); VERHEIJDEN GIJSBERTUS) 9 May 1996 (1996-05-09) abstract page 4, line 15 Seq Id No 6 claims	1,13-16

1

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INTERNATIONAL SEARCH REPORT

...formation on patent family members

Inter ·nal Application No
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